Amendments To The Claims

The listing of claims will replace all prior versions and listings of claims in this application.

Listing of Claims:

Claims 1-34 (canceled)

- 35. (currently amended) A method for producing a [human-like] <u>humanized</u> glycoprotein in a lower eukaryotic host cell that does not display a 1,6 mannosyltransferase activity with respect to the N-glycan on a glycoprotein, the method comprising the step of introducing into the host cell [one or more enzymes for production of a Man₅GlcNAc₂ carbohydrate structure, wherein at least 30% Man₅GlcNAc₂ is produced within the host cell which can serve as a substrate for GnT1 *in vivo*] a hybrid mannosidase enzyme comprising:
- (a) a catalytic domain selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in the subcellular location where the domain is targeted; and
- (b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to a subcellular location where the domain will exhibit optimal activity;

whereby in excess of 30 mole % of the N-glycan structures have a Man₅GlcNAc₂ structure that can serve as a substrate for GlcNAc transferase I *in vivo*.

36-38 (canceled)

- 39. (currently amended) The method of claim 35, wherein [at least one introduced enzyme] the mannosidase is targeted to [the endoplasmic reticulum,] the early, medial, late Golgi or the trans Golgi network of the host cell.
- 40. (currently amended) The method of claim [39] 35, further comprising the step of introducing into the host cell [wherein at least] one or more additional [of the] enzymes [is] selected from the group consisting of mannosidases, glycosyltransferases and glycosidases.
 - 41. (canceled)
- 42. (currently amended) The method of claim 35 or 40, wherein the glycoprotein comprises N-glycans having fewer than six mannose residues.
- 43. (currently amended) The method of claim 35 or 40, wherein the glycoprotein comprises N-glycans having fewer than four mannose residues.
- 44. (currently amended) The method of claim 35 or 40, wherein the glycoprotein comprises one or more sugars selected from the group consisting of N-acetylglucosamine, galactose, sialic acid, and fucose.
- 45. (currently amended) The method of claim 35 or 40, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.
- 46. (currently amended) The method of claim 35 or 40, wherein the host is selected from the group consisting of *Pichia pastoris*, *Pichia finlandica*, *Pichia*

trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia sp., Saccharomyces cerevisiae, Saccharomyces sp., Hansenula polymorpha, Kluyveromyces sp., Candida albicans, Aspergillus nidulans, and Trichoderma reesei.

- 47. (currently amended) The method of claim 35 or 40, wherein the host is deficient in the activity of one or more enzymes selected from the group consisting of mannosyltransferases and phosphomannosyltransferases.
- 48. (previously presented) The method of claim 47, wherein the host does not express an enzyme selected from the group consisting of 1,6 mannosyltransferase; 1,3 mannosyltransferase; and 1,2 mannosyltransferase.
- 49. (currently amended) The method of claim 35 or 40, wherein the host is an OCH1 mutant of *P. pastoris*.
- 50. (currently amended) The method of claim 35 or 40, wherein the host expresses one or more enzymes selected from: GnTI; a UDP-specific diphosphatase; a GDP-specific diphosphatase; and a UDP-GlcNac transporter.
 - 51. (canceled)
- 52. (currently amended) The method of claim 35 or 40, further comprising the step of isolating the glycoprotein from the host.

- 53. (previously presented) The method of claim 52, further comprising the step of subjecting the isolated glycoprotein to at least one further glycosylation reaction *in vitro*, subsequent to its isolation from the host.
- 54. (currently amended) The method of claim 35, further comprising the step of introducing into the host cell [a] nucleic acid [molecule] molecules encoding one or more enzymes for production of the [Man₅GlcNAc₂ carbohydrate structure] <u>humanized</u> glycoprotein selected from the group consisting of mannosidases, glycosyltransferases and glycosidases.

55-56 (canceled)

- 57. (currently amended) The method of claim [56] <u>35</u>, wherein the mannosidase enzyme has optimal activity at a pH between [5.9 and 7.5] <u>5.1 and 8.0</u>.
- 58. (currently amended) The method of claim [56] 35, wherein the mannosidase enzyme [is] comprises an α -1,2-mannosidase catalytic domain derived from mouse, human, Lepidoptera, Aspergillus nidulans, Xanthomonas manihotas or Bacillus sp.
- 59. (currently amended) The method of claim 54, wherein at least one of the [enzyme] enzymes for production of the humanized protein is localized by forming a fusion protein between a catalytic domain of the enzyme and a cellular targeting signal peptide.
- 60. (previously presented) The method of claim 59, wherein the fusion protein is encoded by at least one genetic construct formed by the in-frame ligation of a DNA

fragment encoding a cellular targeting signal peptide with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.

- 61. (currently amended) The method of claim [54] <u>59</u>, wherein the catalytic domain encodes a glycosidase or glycosyltransferase that is derived from a member of the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, Fucosyltransferase and ST, and wherein the catalytic domain has optimal activity at a pH between 5.1 and 8.0.
- 62. (previously presented) The method of claim 54, wherein the nucleic acid molecule encodes one or more enzymes selected from the group consisting of UDP-GlcNAc transferase, UDP-galactosyltransferase, GDP-fucosyltransferase, CMP-sialyltransferase, UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, CMP-sialic acid transporter, and nucleotide diphosphatases.
- 63. (previously presented) The method of claim 54, wherein the host expresses GnTI and a UDP-GlcNac transporter.
- 64. (previously presented) The method of claim 54, wherein the host expresses a UDP- or GDP-specific diphosphatase.
- 65. (new) The method of claim 40, wherein the one or more additional enzymes is targeted to the endoplasmic reticulum, the early, medial or late Golgi, or the trans Golgi network of the host cell.

- 66. (new) The method of claim 65, wherein the one or more additional enzymes is targeted by means of a cellular targeting signal peptide not normally associated with the enzyme.
- 67. (new) The method of claim 40, wherein the one or more additional enzymes is selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes where the enzyme is localized.
- 68. (new) The method of claim 54, wherein at least one nucleic acid molecule encoding one or more enzymes is introduced into the host cell by integration into the host cell chromosome.
- 69. (new) The method of any one of claims 40, 54 or 68, wherein at least one of the encoded enzymes is GnTI.
- 70. (new) A method for producing a humanized glycoprotein in a lower eukaryotic host cell that produces glycoproteins having N-glycan structures wherein an excess of 30 mole % of the N-glycan structures produced within the host cell have a Man₅GlcNAc₂ structure that can serve as a substrate for GlcNAc transferase I *in vivo*, the method comprising the step of expressing in said host cell a hybrid GlcNAc transferase I enzyme comprising:
- (a) a catalytic domain selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in the subcellular location where the domain is targeted; and

- (b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to a subcellular location where the domain will exhibit optimal activity.
- 71. (new) A method for producing a human-like glycoprotein in a lower eukaryotic host cell that does not display alpha-1,6 mannosyltransferase activity with respect to the N-glycan on a glycoprotein, the method comprising the step of introducing into the host cell a hybrid N-acetylglucosaminyl transferase enzyme comprising:
- (a) a catalytic domain selected to have a pH optimum within 1.4 pH units of the average pH optimum of glycosylation-related enzymes in the subcellular location where the domain is targeted; and
- (b) a cellular targeting signal peptide not normally associated with the catalytic domain selected to target the catalytic domain to a subcellular location where the domain will exhibit optimal activity.
- 72. (new) The method of claim 71, further comprising the step of introducing into the host cell a nucleic acid encoding a UDP-GlcNAc transporter.
- 73. (new) The method of any one of claims 35, 40 or 54, further comprising the step of analyzing a glycosylated protein or isolated N-glycan produced in the host cell by one or more methods selected from the group consisting of: (a) mass spectroscopy such as MALDI-TOF-MS; (b) liquid chromatography; (c) characterizing cells using a fluorescence activated cell sorter, spectrophotometer, fluorimeter, or scintillation counter; (d) exposing host cells to a lectin or antibody having a specific affinity for a desired oligosaccharide

Appln No. 09/892,591 Amendment and Reply to Office Action dated July 12, 2004 Office Action dated March 12, 2004

moiety; and (e) exposing cells to a cytotoxic or radioactive molecule selected from the group consisting of sugars, antibodies and lectins.